

Nonhuman Primates: A Critical Role in Current Disease Research

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Abstract

This review article emphasizes the critical role of nonhuman primates (NHPs) in biomedical research. It focuses on the most recent contributions that NHPs have made to the understanding, treatment, and prevention of important infectious diseases (e.g., acquired immunodeficiency syndrome, hepatitis, malaria) and chronic degenerative disorders of the central nervous system (e.g., Parkinson's and Alzheimer's diseases). The close phylogenetic relation of NHPs to humans not only opens avenues for testing the safety and efficacy of new drugs and vaccines but also offers promise for evaluating the potential of new gene-based treatments for human infectious and genetic diseases.

Key Words: AIDS; animal models; gene transfer; hepatitis; malaria; nervous system disorders; nonhuman primates; vaccines

Introduction

Nonhuman primates (NHPs)¹ are excellent models for studying human biology and behavior because of their close phylogenetic relation to humans. Their use in biomedical research is critical to advancements in medical science. The text below describes that vital role and focuses on some of the most recent contributions that NHP models have made to the understanding, treatment, and prevention of certain human diseases and disorders.

In an earlier era, important medical successes such as the discovery of the Rh factor and the development of poliovirus

vaccine demonstrated the importance of using NHPs in biomedical research. At the time of this writing, their use has expanded into virtually every area of medicine, and each year new species are added to the research armamentarium (Bowden and Smith 1992). The literature includes numerous reviews on the use of NHPs in biomedical research (e.g., King and Yarbrough 1995; King et al. 1988; Stone et al. 1987).

Although there are various taxonomic lists, the primate order may be viewed as being made up of (1) prosimians (e.g., lemurs, lorises, tarsiers) and (2) anthropoidia consisting of New World primates (e.g., marmosets, spider monkeys, cebus) and Old World primates further divided into monkeys (e.g., macaques, baboons) and greater and lesser apes (gorillas, chimpanzees, orangutans, and gibbons, respectively). Overall, there are more than 200 species of NHPs, but only about 30 are used in research (NRC 1998; PIN 2000). The total number used each year has remained relatively constant for the past decade, ranging in the mid-50,000s (USDA 2000). NHPs are used very sparingly as surrogates for humans in medical research; they account for only about 0.3% of the animals required by the US research community. Because most studies involving NHPs do not require euthanasia, NHPs may actually account for less than 0.05% inasmuch as fewer animals are needed each year as replacements (Bowden and Johnson-Delaney 1996).

The process for acquiring laboratory primates has evolved significantly over several decades. When the research community first began using primates, the need was met largely through importation. As source countries adopted more rigid regulations and outright prohibition of exports of certain species, it was necessary to meet the problem of supplying enough animals in ways that did not threaten their future existence. With the exception of the importation of certain monkey species, commercial breeding colonies within the United States provide a large part of the NHPs needed for research (Bowden and Smith 1992). In addition, as part of the National Center for Research Resources Primate Program, the eight Regional Primate Research Centers have the responsibility for improving breeding practices and providing biomedical researchers access to NHP resources (Strandberg 2000).

Throughout the biomedical community, there is appreciation for the fact that because NHPs are genetically close to humans, they deserve special consideration, and scientists strive to achieve the greatest human benefit (i.e., reducing or curing disease) with minimal cost to the animals (i.e., pain or distress). In recognition of their intelligence and complex

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¹Abbreviations used in this article: AD, Alzheimer's disease; AIDS, acquired immunodeficiency syndrome; BPD, bronchopulmonary dysplasia; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; L-DOPA, levodopa; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NHP, nonhuman primate; NIAID, National Institute of Allergy and Infectious Disease; RDS, respiratory distress syndrome; RSV, respiratory syncytial virus; SHIV, simian-human immunodeficiency virus; SIV, simian immunodeficiency virus.

psychological, physical, and social needs (Prince and Brotman 2001), efforts are continuing toward the development of new ways to improve the physical environment and promote the psychological well-being of NHPs in laboratory settings (NRC 1998). Indeed, scientists have deemed it important to study the natural history and behavior of NHPs to understand their diverse needs better. In addition, as more information is obtained about the human genome, more efforts are being made to find suitable genetically modified mouse models that may be used in lieu of these higher mammals in an effort to reduce our current dependence on them (Feitelson and Larkin 2001).

AIDS

The acquired immunodeficiency syndrome (AIDS¹) was first recognized in 1981 and has since become a pandemic. To date, the causative agent, human immunodeficiency virus (HIV¹), has infected more than 34 million adults and children worldwide and has claimed more than 18 million lives (Schwartlander et al. 2000). HIV acts by destroying helper CD4+ T-lymphocytes, which are essential for the normal functioning of the human immune system. Although potent antiretroviral drug combinations have reduced the number of AIDS deaths in technically advanced nations, the cost limitations of these therapeutic interventions emphasize the need for a safe and effective vaccine to bring the epidemic under control. Because infected individuals often carry the virus for many years before significant damage to the immune system occurs, scientists believe that a vaccine will help infected persons live longer by priming the immune system to keep HIV replication under control.

Researchers face major challenges in trying to develop a vaccine against AIDS. With the possible exception of survivors who are exposed to HIV but remain disease free, investigators have no human model to guide them. These long-term nonprogressors develop neutralizing antibodies and cellular immune responses that resist HIV infection (NIAID 1997). For the present, success in developing an HIV vaccine depends on studying animals that not only are susceptible to infection but also progress to develop AIDS or AIDS-like symptoms similar to those in humans.

Since the early 1990s, scientists have developed NHP models that can be naturally and experimentally infected with primate lentiviruses (see reviews by Hirsch and Lifson 2000; Nathanson et al. 1999). Various simian immunodeficiency viruses (SIVs¹), the NHP counterparts of HIV, infect many different species of macaque monkeys and cause an AIDS-like syndrome. More recently, simian-human immunodeficiency viruses (SHIVs¹), genetically engineered chimeras composed of an SIV core enclosed in an outer coat envelope protein of HIV, have revealed advantages over the SIV model. Many of these viruses retain the ability to cause CD4+ T-cell lymphopenia and an AIDS-like disease in chimpanzees and several monkey species; however, they express human surface proteins, which make them valuable as challenge viruses

for evaluating HIV envelope-based vaccines (Reimann et al. 1996).

Although progressive infection with HIV-1 can occur in some chimpanzees, chronically infected animals usually maintain normal numbers of CD4+ T-lymphocytes and do not become immunodeficient (O'Neil et al. 2000). The one exception stems from a report that a chimpanzee infected with three different isolates of type-1-HIV over a period of 10 yr revealed a persistent decline in CD4+ T-lymphocytes that progressed to AIDS or an AIDS-like disease. Blood from this animal that was transfused into an uninfected chimpanzee induced a rapid depletion of CD4+ T-lymphocytes but did not cause clinical disease (Novembre et al. 1997). Without disease as an endpoint, researchers can measure only the infection-blocking effect of candidate vaccines.

Using NHP models, scientists are exploring a number of different approaches to develop vaccines that either generate antibody-related immunity produced by B-lymphocytes or cell-mediated immunity produced by cytotoxic T-lymphocytes, or both. Examples of immunogens currently being tested in NHP models include recombinant viral subunit (peptide fragment) vaccines, formalin-inactivated whole virus vaccines, attenuated virus vector vaccines carrying the gene for an HIV protein, and plasmid DNA vaccines containing genes encoded for HIV protein. However, because AIDS is a complicated disease involving many molecular events in several different cell types, a vaccine that works in NHPs may not work in humans. Current efforts are being directed not toward development of an actual vaccine but instead toward vaccine strategies and a greater understanding of the basic biology of SIV/HIV. In fact, the latest strategy, to prevent attachment of HIV to cells by using blocking agents, is being tested in humans. For a survey of ongoing research on immunization strategies, see Baltimore and Heilman 1998; Hirsch and Lifson 2000; and Nathanson and Mathieson 1999, 2000.

In testing a gene transfer strategy, researchers removed the CD4+ T-lymphocytes from monkeys and successfully inserted an antisense oligonucleotide complementary to viral SIV-RNA, which degrades SIV-RNA and blocks the production of two regulatory proteins (tat and rev) essential for viral replication. The return of the engineered lymphocytes and subsequent challenge with SIV revealed that the procedure enabled the monkeys to maintain normal levels of CD4+ T-lymphocytes and significantly reduce the viral burden (Donohue et al. 1998). (For a review of additional gene transfer strategies to inhibit HIV, see Morgan 1999.)

Recently, a team of researchers reported that rhesus monkeys vaccinated with the SIV-tat protein produced a killer T-cell response that contained the SIV infection (Allen et al. 2000). Another group has confirmed that the tat protein may be an important factor in acquired immunity to AIDS. They showed that immunizations with a chemically inactivated tat (toxoid) inhibits viral replication after challenge with SHIV89.6P, a highly pathogenic virus, and prevents CD4+ T-lymphocyte decline (Cafaro et al. 2000; Pauza et al. 2000).

In 1999, the National Institute of Allergy and Infectious

Disease (NIAID¹) convened a group of virologists, primatologists, veterinarians, and animal protectionists to discuss the pros and cons of inoculating chimpanzees with a virulent strain of HIV that could cause fatal disease. (For a more detailed account of this meeting, see Cohen 1999a.) Some scientists favored continuing acute terminal studies in chimpanzees as long as the outcome would lead to a model for testing human candidate vaccines. However, even with the potential success of the model, the meeting participants expressed concern about the ethics of using chimpanzees for this research and strongly urged that studies with monkey models be given preference.

Hepatitis

A detailed review of the role of NHPs in the study of hepatitis is beyond the scope of this article, and readers are urged to consult the other contributions in this issue of *ILAR Journal* (Beames et al. 2001; Feitelson and Larkin 2001; Gale and Beard 2001; Gerin 2001; Lanford et al. 2001; Prince and Brotman 2001; Purcell and Emerson 2001; Strader and Seeff 2001; Tennant and Gerin 2001). Nevertheless, the following brief information is provided to supplement the literature.

Hepatitis C

Hepatitis C Virus (HCV¹) is an enveloped RNA virus that causes acute and chronic liver disease. A recent study indicates that the prevalence of chronic hepatitis infection is endemic in most parts of the world. HCV has infected an estimated 3.9 million people in the United States and 170 million worldwide. People who use illegal drugs or engage in high-risk sexual behavior account for most infections. The advent of a screening test for detecting antibodies to HCV has virtually eliminated blood transfusions as a source of infection (Alter and Seeff 2000).

Most people harbor HCV without clinical symptoms for many years before they develop serious complications such as cirrhosis and liver cancer (reviewed by Strader and Seeff 2001). Approximately 15 to 25% of infected persons can clear the virus from their bloodstream within several weeks, whereas the remaining 75 to 80% develop a chronic infection (Cohen 1999b). Treatment with interferon alone, or combined with the antiviral drug ribavirin, is not highly efficacious and can cause significant side effects (Abrignani et al. 1999; Strader and Seeff 2001). Consequently, there is a pressing need to develop vaccines aimed at preventing HCV transmission and eliminating chronic infection.

In the absence of a reliable tissue culture system and a small animal model, a great deal of what is known about the nature of HCV infections has been learned through studies with chimpanzees—the only known nonhuman host for HCV. Chimpanzees can be infected with HCV and treated with potential antiviral drugs. They can also be immunized with various viral vaccines and challenged with HCV isolates

to validate potential targets for the development of antiviral interventions (Abrignani et al. 1999; Kolykhalov et al. 2000).

A major breakthrough has been the successful construction of full-length, functional cDNA clones of HCV. The RNA transcripts of these clones initiated infection and produced disease when injected directly into the liver of chimpanzees (Kolykhalov et al. 1997; Yanagi et al. 1997; reviewed by Gale and Beard 2001; Lanford et al. 2001). The virus clones now can be biologically amplified and used as challenge inocula in chimpanzees to develop a better understanding of the molecular biology and natural history of HCV infection.

The relation of viral persistence, the immune response to E1 and E2 envelope proteins, and the sequence variability in E1 and E2 envelope genes of HCV have been examined in chimpanzees (reviewed by Gale and Beard 2001; Lanford et al. 2001). Viral clearance was not associated with antibodies to the E1 or E2 envelope proteins (Bassett et al. 1999). Two recent reports suggest that the ultimate outcome of an HCV infection may depend on a host's ability to mount a strong cellular and humoral immune response in the early phase of primary infection. One study conducted in chimpanzees demonstrated that HCV persistence is related to changes in the hypervariability region (HVR1) of the putative E2 envelope gene of HCV (Ray et al. 2000). This finding was later confirmed in human studies (Farci et al. 2000). These investigators also found that successful resolution of hepatitis in the acute stage is associated with little or no evolutionary change in viral surface proteins (viral sequences), whereas chronic hepatitis was almost exclusively correlated with the production of variants of the E2 gene in the HVR1 region of the virus. In both studies, the data suggest that the development of viral variants or quasispecies during the early phase of HCV infection predict whether the infection will be resolved or become chronic.

The results of an investigation into the type of immunity responsible for the resolution of HCV infection suggest that cytotoxic T-lymphocytes are better correlated with immunity than antibodies (Cooper et al. 1999). One group of scientists has presented preliminary evidence that an HCV vaccine consisting of recombinant envelope proteins elicits neutralizing antibodies and stimulates the production of CD4+ cytotoxic T-lymphocytes during the acute phase, preventing chronic infection in chimpanzees (Abrignani et al. 1999). Another group has reported that immunization with E2 glycoproteins and peptides from the E2 hypervariable region of the virus is effective in chimpanzees (Esumi et al. 1999).

Hepatitis B

Hepatitis B virus (HBV¹) is also transmitted by contact with an infected person's blood or bodily fluids, mostly through the use of shared needles and high-risk sexual behavior. HBV infection produces a transient viremia that lasts about 4 to 8 wk or becomes chronic. In 90% of infected people, the

acute infection is clinically silent or produces flu-like symptoms, and the remaining 10% develop a chronic infection with a high risk of cirrhosis and hepatocellular carcinoma. Although vaccines generally became available in 1982, the infection and its sequelae remain a major cause of morbidity and death. Globally, close to 3 billion people are infected, 350 million become chronic carriers, and 1 to 1.5 million die each year. If the vaccine were more widely used, most cases of the disease could be prevented (NIDCR 1998).

Hepatitis B viruses are found in a wide variety of mammals and birds (Hu et al. 2000; Tennant and Gerin 2001). However, chimpanzees, and to a lesser extent rhesus monkeys, are the only animals that can be infected by human HBV genotypes. Although they are not considered the natural host for the various HBV subtypes, chimpanzees develop circulating antibodies and increased enzyme levels indicating liver damage after virus inoculation (Barker et al. 1975). Chimpanzees also mount cellular immune responses to HBV, similar to those observed in acutely infected humans (Bertoni et al. 1998).

Approximately 10% of young adults fail to respond to the commercially available HBV vaccine, prompting the demand for a vaccine that can stimulate strong cellular and humoral immune responses. Using chimpanzees, one group of researchers is testing a new recombinant vaccine that encodes for three viral surface proteins that stimulate strong cellular and humoral immune responses (Pride et al. 1998). Other groups have shown that an adjuvant (an oligodeoxynucleotide-CpG) increases seroconversion and produces significantly higher titers of antibody when administered with the HBV commercial vaccine (Davis et al. 2000; Hartman et al. 2000).

Recently, a study conducted in chimpanzees has advanced our understanding of how the body protects against the spread of HBV without harming the liver. Researchers have shown that during a typical infection, elimination of HBV-DNA is accomplished by a tissue-sparing process rather than by the destruction of infected hepatocytes (Guidotti et al. 1999; Prince and Brotman 2001).

Malaria

The World Health Organization estimates that 300 to 500 million new cases of malaria occur each year, claiming two to three million lives worldwide. In many developing countries, and especially in sub-Saharan Africa and Southeast Asia, malaria is endemic. Because of the increase in international travel, imported cases are becoming more common in the United States (WHO 1998).

In the last decade, considerable progress has been made in the development of malaria vaccines. More than 30 distinct antigens in the various cycle stages of the parasite have been proposed as potential vaccine candidates (James and Miller 2000). Owl and squirrel monkeys are excellent models for testing vaccines because they can be infected with the two *Plasmodium* species (*P. falciparum*, *P. vivax*) that cause most human disease. The owl monkey is the primate model

recommended by the World Health Organization for testing vaccines (WHO 1998). The vast majority of vaccine studies, including those reported below, have used this primate species in developing immunizing formulations. Chimpanzees occasionally are used to produce infective parasites (sporozoites) for monkey challenge studies (Sullivan et al. 1996).

Malarial parasites (protozoans) from the genus *Plasmodium* cause disease in many mammalian species, including humans, apes, monkeys, rodents, and birds. However, of the more than 100 *Plasmodium* species, only four infect humans. *Plasmodium falciparum* causes the greatest number of deaths and life-threatening complications (NIAID 2000). Although malaria can be controlled with insecticides that kill the mosquito vector and with antimalarial drugs, the organisms are becoming increasingly resistant to treatment. The development of effective vaccines and new drugs is imperative in the years ahead (WHO 1998).

Currently, vaccines are being developed in NHPs to prevent the emergence and spread of malaria in the community and to reduce the severity of the disease and the risk of death in infected individuals (Carter et al. 2000). Researchers have been successful in identifying a number of surface proteins of the mosquito-stage parasite as suitable target antigens for transmission blocking vaccines (Carter et al. 2000; Kaslow 1997), and candidate blocking vaccines are now being tested in human phase I safety and immunogenicity studies. Other investigators are evaluating candidate vaccines composed of synthetic peptides, multiple antigenic peptides, or recombinant proteins derived from the blood stages of *P. falciparum*. These vaccines have been shown to be immunogenic in monkeys and chimpanzees, especially when they are administered with immune enhancers such as oligodeoxynucleotides (Benmohamed et al. 2000; Egan et al. 2000; Holder et al. 1999; Jones et al. 1999; Kumar et al. 2000; Moreno et al. 1999). In addition, other scientists are attempting to develop multiantigen, multistage vaccines based on recombinant protein antigens of *P. falciparum* strains (James and Miller 2000). These vaccines are directed at preventing the invasion of hepatocytes or erythrocytes.

Respiratory Syncytial Virus

Human respiratory syncytial virus (RSV¹) is the most common cause of lower respiratory tract infections in infants, young children, and the elderly. Although RSV may produce only cold-like symptoms and bronchiolitis in healthy persons, it can cause life-threatening pneumonia leading to hospitalization in premature infants and in children and adults who are otherwise immunocompromised, such as AIDS patients. Each year, RSV contributes significantly to deaths worldwide, including an estimated 4500 in the United States. To date, there is no licensed vaccine available to prevent RSV infection (CDC 1996).

There is a critical need to establish reliable and well-defined animal models for the *in vivo* testing of RSV vaccines. Studies in nonprimate models cannot duplicate all

forms of RSV disease (Byrd and Prince 1997). To date, the chimpanzee is the only experimental animal that is susceptible to RSV infection and develops a respiratory illness similar to the disease seen in human infants. Consequently, chimpanzees have become the model of choice for studying RSV disease and for evaluating live virus vaccine candidates against the disease. Chimpanzees used in these studies must be relatively young (less than 2 yr of age) before they develop protective antibodies to RSV infections (Belshe et al. 1978).

Although a great deal of information about the molecular biology and pathogenesis of the cloned virus is known, the RSV-induced immune response is poorly understood. Studies in animals and humans have shown that neutralizing antibodies can protect against RSV infection and illness (Hemming et al. 1995). Maternal antibody levels also correlate with the protection of infants. Because the virus causes severe disease in early infancy, a vaccine also must be effective in the presence of maternal antibody (Murphy et al. 1994).

The technology exists to prepare live infectious RSV from cDNA, making it possible to design a recombinant RSV vaccine with known attenuating insertions, which is suitable for intranasal administration (Collins et al. 1999). The safety and efficacy of such vaccines can then be evaluated in seronegative chimpanzees. Recently, several candidate vaccines—a cold-passaged, temperature-sensitive RSV subgroup B (Crowe et al. 1999), a live attenuated chimeric virus (Whitehead et al. 1999), and viral subunits of attenuated strains formulated with purified fusion and attachment proteins (Hancock et al. 2000)—were shown to be immunogenic and to confer high levels of resistance on chimpanzees. Some of these live vaccines are being evaluated, or soon will be, in humans.

Periodontitis

Periodontal or gum disease is the most common cause of bone and tooth loss in humans. Oral infections caused by bacteria that colonize the tooth surface and gingiva initiate the destruction of enamel, dentin, root surfaces, and the components of the periodontium (Graves et al. 1998). Periodontal tissue destruction also creates a route for bacterial pathogens to enter the blood stream.

Periodontitis is also a common health problem for several captive NHP species. Macaque monkeys (*cynomolgus*) develop dental calculus, plaques, and associated gingivitis, which often progress into periodontal disease (Schou et al. 1993, 1996). Experimental chronic gingivitis and temporary periodontitis, with subsequent destruction of bone, can be readily induced and reversed in these monkeys (Wirthlin and Hussain 1992). This species also manifests a subgingival flora characteristic of the anaerobic Gram-negative bacteria found in human periodontal pockets (Persson et al. 1994a).

NHP models allow scientists to manipulate variables in ways not possible in humans when studying the changes that occur in the progression of early gingivitis to periodontitis. Several reports describe the cellular events that occur in

inflamed gingival connective tissue and dental alveoli, the bony sockets in the jaws of *cynomolgus* monkeys. Investigators also have measured specific changes in the levels of bacterial by-products, acute phase biomarkers such as cytokines and interleukins, and serum lipids and lipoproteins associated with the inflammatory process (Assuma et al. 1998; Ebersole et al. 1999). Using this model, these scientists have found evidence that the cluster of changes described above may be associated with increased risk of atherogenesis (i.e., the formation of lesions in arterial walls). Some investigators now believe that periodontitis may be an important risk factor for, or may be linked to, the initiation of the atherosclerotic process, which can lead to coronary heart disease (Beck et al. 1998; Offenbacher et al. 1999; Page 1998).

The efficacy and safety of vaccines against periodontitis, composed of bacterial immunogens prepared from the animal's own subgingival microbial flora, are being assessed in NHPs with the aim of slowing or halting the progression of periodontal tissue destruction and protecting against bone loss (Cox et al. 1997; Giardino et al. 1996; Holt et al. 1995; Houston et al. 1999; Persson et al. 1994b). It is important to select the bacterial immunogens that elicit protection from periodontal tissue destruction. However, the insights obtained from these studies should help optimize the potential for immunological interference with progressing periodontitis. Investigators also are testing the effect of certain food additives that can be placed in the normal primate diet to retard calculus formation and reverse or prevent subsequent development of periodontal disease (Brady et al. 2000).

Bronchopulmonary Dysplasia and Respiratory Distress Syndrome

Bronchopulmonary dysplasia (BPD¹) is a disabling and sometimes fatal chronic lung disease of newborn infants resulting from the abnormal development of the lungs. It occurs most often in premature babies who require mechanical ventilation and oxygen under pressure to survive respiratory distress syndrome (RDS¹) or hyaline membrane disease. RDS is caused by a deficiency or dysfunction of pulmonary surfactant, a substance produced by alveolar cells that coats the inner surface of the lungs. The surfactant acts by lowering the surface tension at the air-cell interface to promote the uptake of oxygen and excretion of carbon dioxide in expired air. RDS together with BPD account for much of the infant morbidity and mortality in the United States, with up to 10,000 cases of BPD occurring each year in surviving infants (NHLBI 1998; Soll 2000).

Research with premature baboons has provided important information about lung growth and development in long-term survivors with BPD that could not have been obtained from studies with human babies. Extremely immature baboons treated with exogenous surfactant and oxygen ventilation develop chronic lung disease that is very similar, if not identical, to chronic lung disease in infants. Moreover,

baboons can be studied in a controlled environment where they are bred to produce pregnancies of known gestational age and where the results are not confounded by variable causes of premature delivery (deLemos and Coalson 1992).

The results of studies in the preterm baboon have provided important basic findings and practical therapeutic information to decrease the severity of, or prevent, BPD. Various studies have shown that chronic lung injury resulting from high ventilation and high inspired concentrations of oxygen is associated with diffuse alveolar damage and abnormal surfactant metabolism and function (Awasthi et al. 1999; Coalson et al. 1988, 1995; Seidner et al. 1998). Administration of both natural and synthetic surfactants improves the clinical outcome of RDS and decreases the progression of BPD in premature baboons (Huang et al. 1995; Maeta et al. 1988, 1990). The exact causes of BPD remain under investigation; however, studies in baboons are expected to complement human research and may ultimately lead to improved clinical management and greater survival for infants with these respiratory conditions.

Gene Transfer

As the result of remarkable advances in recombinant DNA technology and the recently completed mapping of the human genome, human genetic engineering or gene transfer experiments are currently being investigated to provide alternative modalities for managing and correcting a number of inherited metabolic, infectious, and malignant diseases that are not amenable to current approaches. Gene transfer involves incorporating a gene into an individual either by the administration of naked DNA or DNA complexed to a harmless virus that carries genetic material into a target somatic cell genome, or by the introduction of cells manipulated to harbor foreign DNA. Because the genetic material is the potential therapeutic agent, the recipient's cells can be modified to restore or enhance a normal activity, to provide a new or changed activity, or to prevent the expression of an endogenous disease-causing gene. Successful therapy would require efficient transfer, faithful integration of unrelated DNA into a host genome, and subsequent expression. (For a more complete discussion of this topic, see NIH 1995.)

Virtually all normal biological processes and diseases have a genetic component. Some disorders such as sickle cell anemia involve single genes, and other conditions such as cardiovascular disease involve multiple genes and environmental factors. Because of unique safety, social, and ethical concerns, it is not possible to carry out all of the genetic manipulations needed to design and evaluate gene transfer strategies in human patients. Preclinical safety tests must be addressed in primates and other mammals before moving this technology into clinical practice (Morgan and Anderson 1992; Stone et al. 1987).

Until now, most of the research aimed at understanding and treating genetic disease has been conducted in naturally occurring or genetically altered animal models that mimic

human disease. The recent completion of the Human Genome Project and the partial mapping of other mammalian genomes, including mice, rats, chimpanzees, and baboons, has led to the identification of genes for many human conditions, including those with animal counterparts. Because genetic conservation among mammalian genomes is extremely high, the mouse and the rat have been, and will continue to be, the most practical models for understanding disease processes and testing gene therapies (Davisson 1999; Kwik-Black 2000).

Nonetheless, a single mammalian model cannot mimic all human diseases, and additional models with similar phenotypes that more accurately reflect corresponding human disorders are needed. The close evolutionary relation of humans and NHPs argues strongly for the use of NHPs in certain preclinical studies. Now that the sequencing of the human genome has been achieved, there is a compelling argument for sequencing the genomes of other mammals, in addition to the mouse. The obvious candidates are the chimpanzee and the rhesus macaque (McConkey and Varki 2000; Rogers and VandeBerg 1998). These models can be expected to yield the most valid assessment of the potential benefits and risks of gene transfer systems in humans.

Gene transfer experiments are being actively pursued in NHPs to develop alternative treatments for a number of human infectious and genetic diseases. In anticipation of an explosion of gene-based treatments in the next decade, it is important to emphasize the unique role of NHPs in evaluating the safety of these methods.

Brain Disorders

NHPs are extremely valuable models for investigating the functions of the normal brain as well as brain-related disorders and disabilities. Annually, more than 90 million people in the United States are afflicted with brain-related conditions, which include mental, neurological, and addictive disorders. Yet about 90% of what is known about the brain today has been learned from animal studies in the last 10 yr (Judd 2000).

In general, the approach to understanding the brain and its cognitive abilities requires collecting and analyzing experimental data from the functioning central nervous system of active animals while the brain performs standard mental operations. The NHP model allows researchers to conduct combined behavioral, physiological, and anatomical studies that could not be carried out in humans (Newsome and Stein-Aviles 1999).

The information gained from primate research is directly applicable to the human condition because NHPs share many features of brain biology and physiology with humans. Old and New World monkeys and many species of small primates are the animal models of choice for neurological studies because many of the associated regions of the cerebral cortex of these species are present and identifiable. Studies using NHPs have provided a more precise map of the circuitry and

functions of the cerebral cortex than could ever have been achieved using other species, including humans (Goldman-Rakic 1996).

Monkeys can be trained to perform a wide variety of simple cognitive tasks involving perception, attention, and decision-making in return for positive rewards. While carrying out these tasks, the electrical activity of individual nerve cells can be measured and mapped using small microelectrodes positioned at specific locations within the brain. Microelectrodes are inserted through a penetrable window in the cranium that had been implanted earlier under anesthesia; and once in place, the electrodes cause no pain because the brain lacks pain sensors. Single-cell recording in awake, behaving animals is perhaps the most powerful approach to understanding the neural basis of all primate behavior (Goldman-Rakic 1999). To complement microelectrode recordings, investigators are beginning to use new non-invasive experimental techniques such as functional magnetic resonance imaging (fMRI) to determine how information is processed in the NHP brain (NEI 1998; Newsome and Stein-Aviles 1999).

The limited space of this article precludes a review of the vast number of primate experiments that have contributed to our knowledge of the biological basis of normal brain function and the abnormalities that occur as models of human diseases. Basic studies of neuroanatomy and neurobiology of the NHP brain have contributed to mapping the circuitry and functions of the cerebral cortex, helping researchers better understand the neural processes that underlie sensory perception, long- and short-term memory, movement, learning, emotion, decision-making, and communication. Studies involving NHP models have yielded many human health-related applications and a better understanding of auditory and visual impairment, learning and memory deficits, substance abuse, spinal cord and brain injury, aberrant social behavior, and mental illness. The ultimate aim of this research is to understand the human brain better.

Parkinson's Disease

Parkinson's disease (PD¹) is the most common progressive neurodegenerative movement disorder of the central nervous system, primarily affecting older individuals (1% of the population over 55 yr). Approximately one million people in the United States have PD, with 50,000 cases reported annually. Victims have pronounced tremors that affect the extremities as well as other physical symptoms, including slow movement, rigidity, postural imbalance, depression, and memory impairment (Young 1999).

The link between PD and the progressive degeneration of dopamine-producing nerve cells in the substantia nigra—a small structure located in the midbrain—was discovered in rabbits and later confirmed in several other animal species, including NHPs. Dopamine is a chemical that helps transmit signals to other nerve cells. This finding has led to the

treatment of PD with levodopa or L-DOPA,¹ which is converted into dopamine within the blood-brain barrier. Although the administration of L-DOPA remains the treatment of choice, the effects are not sufficiently lasting and lead to the development of dyskinesia, a condition characterized by spontaneous or uncontrolled movements. Therefore, it remains critical to find new classes of drugs or other ways to treat PD and drug-induced dyskinesias, the development of which will likely require primates as models to understand the cause and progression of neurodegeneration (reviewed by Tolwani et al. 1999).

In the 1980s, the accidental observation that users of synthetic heroin developed PD from a contaminating substance (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine or MPTP¹) opened new avenues for PD research. The administration of MPTP induces dopamine depletion in a wide variety of animal species (Tolwani et al. 1999). However, the behavioral changes induced in the MPTP-treated NHPs closely resemble those of the human disorder. Researchers have found that MPTP selectively destroys the substantia nigra in monkeys, marmosets, and baboons and produces the cardinal signs of parkinsonism. These NHP models are unique because no other mammalian species shows a resting tremor after destruction of the substantia nigra. In addition, MPTP-treated primates readily develop L-DOPA-induced dyskinesias that are virtually indistinguishable from those in human patients with PD (Burns et al. 1983).

Because the complexity of the central motor pathways of the primate brain closely resembles that of humans, NHPs have played a critical role in the development of effective and safe therapeutic interventions to slow or halt disease progression. These studies are directed at either developing strategies for prolonging and enhancing the effects of L-DOPA or for reducing or preventing dyskinesia from chronic L-DOPA treatment. Researchers are also evaluating a number of novel intervention strategies in MPTP-treated primate models. These strategies include determining the optimal dose and timing of L-DOPA administration to ameliorate cognitive deficits (Fernandez-Ruiz et al. 1999), evaluating alternatives to L-DOPA treatment (Andringa et al. 1999), developing new drugs (agonists) to reverse motor deficits or prevent dyskinesias (Goulet and Madras 2000; Henry et al. 1999; Pearce et al. 1999), transplanting allogenic fetal and embryonic neural cells to provide a supply of dopamine-producing cells (Bakay et al. 1998), implanting polymer-encapsulated dopaminergic cells (Lindner and Emerich 1998; Yoshida et al. 1999), creating genetically engineered cells or using harmless viral vectors to add functioning genes that provide a continuous supply of dopamine (During et al. 1998; Kordower et al. 1999), and relieving symptoms using high-frequency brain stimulation (Benabid et al. 2000) or by surgical or chemical ablation of the globus pallidus (Lieberman et al. 1999). Researchers are just beginning to study factors that influence the onset and persistence of dyskinesia induced by L-DOPA (Jenner 2000; Langston et al. 2000).

Alzheimer's Disease

Alzheimer's disease (AD¹) is an irreversible, progressive brain disorder that affects nearly 4 million persons in the United States and more than 20 million worldwide. At the current rate, the number of US patients with this disease could increase to 10 million by 2025. Approximately 10% of persons over the age of 65 are diagnosed with AD, as are nearly 50% of those older than 85 yr. The annual cost of caring for AD victims and their families is estimated at \$50 billion (NIA 1999).

There is a close correlation between the chief symptoms of AD and the cerebral accumulation of plaques containing beta-amyloid protein. The cholinergic brain cells of AD patients undergo a cycle of destruction that reduces the choline concentration in proportion to the beta-amyloid concentration. This process, in turn, leads to a decrease in the production of acetylcholine, a neurotransmitter that mediates cellular communication and contributes to the storage and retrieval of memory (Galdzicki et al. 1994).

Since the early 1990s, extensive studies in macaque monkeys have laid the groundwork for identifying the critical regions of the brain that are essential for memory and cognition—structures in the medial temporal lobe of the brain, the hippocampus, and several closely associated regions of the prefrontal cortex (Squire and Zola-Morgan 1991). Aging monkeys also develop beta-amyloid plaques with neuronal loss and corresponding cognitive deficits like those seen in humans (Merrill et al. 2000). The beta-amyloid protein is also identical to that in humans (Pawlik et al. 1999). Monkeys can be trained to perform certain memory-related tasks that permit the evaluation of changes in cognitive memory and emotional behavior during aging. Various treatment and prevention strategies can then be tested in these animals for their potential to enhance behavioral performance (Teng et al. 2000).

The only drugs approved for the treatment of AD are directed at boosting memory deficits. Cholinergic drugs are being tested to improve task performance in aged rhesus monkeys (Buccafusco et al. 1999; Callahan 1999; Harder and Ridley 2000). An exciting new development is a possible vaccine for AD. Mice treated with a vaccine composed of a synthetic protein fragment from beta-amyloid plaques, called AN-1792, not only kept beta-amyloid from accumulating but also eliminated pre-existing deposits in the brain. Scientists believe that removal of the plaques could relieve the symptoms of AD. The vaccine was well tolerated when tested in several animal species, including monkeys and humans (Schenk et al. 2000).

Conclusion

NHPs play a key role in our quest to understand better and treat many diseases and disorders. There is little question that research in NHPs has extended our fundamental knowledge of human diseases and accelerated the clinical develop-

ment of promising new vaccines and drugs. We marvel at the breadth and depth of current biomedical research that is benefiting so greatly from the use of NHP models—from infectious diseases to degenerative diseases and disorders of the central nervous system.

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